STUDIES ON THE ETIOLOGY OF HEARTWATER.

I. Observation of a Rickettsia, Rickettsia ruminantium (N. Sp.), in the Tissues of Infected Animals.*

By E. V. COWDRY.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

Plates 10 and 11.

(Received for publication, January 26, 1925.)

Heartwater is defined by Spreull[1] as "a specific febrile disease affecting sheep, goats and cattle in South Africa and due to an ultra-visible virus transmitted by the bont tick—*Amblyomma hebræum* (Koch)."

The name "heartwater" suggests the most characteristic lesion usually met with at autopsy; namely, hydropericardium. According to Hutcheon[2] the disease was first noticed in South Africa in 1860 coincident with the appearance of the bont tick, *Amblyomma hebræum*.[3] Owing to the severe economic losses which it has entailed it has since been the subject of much experimentation by Hutcheon[4] and others. Edington[5] was the first to transmit heartwater to susceptible cattle by the inoculation of blood containing the virus. In the same year, 1899, Louns-

---

*First contribution by the South African Expedition of The Rockefeller Institute for Medical Research.

The experiments were made in the Laboratory of the Department of Agriculture at Onderstepoort and cordial thanks are due to the Government of the Union of South Africa, to Sir Arnold Theiler, and to the members of his staff for the many courtesies extended.

[1] Spreull, J., *Agric. J. Cape of Good Hope*, 1904, xxiv, 433; *J. Agric. Union of South Africa*, 1922, iv, 236.

[2] Hutcheon, D., *Agric. J. Cape of Good Hope*, 1900, xvii, 410.

[3] Heartwater has since been reported in Angola and the Belgian Congo (Van Saceghem, R., *Bull. Soc. path. exot.*, 1918, xi, 423).

[4] Hutcheon, D., *Agric. J. Cape of Good Hope*, 1901, xix, 302; 1902, xx, 633; 1903, xxii, 438.

[5] Edington, A., *Agric. J. Cape of Good Hope*, 1904, xvii; *J. Comp. Path. and Therap.*, 1904, xvii, 141; *Rep. Director Gov. Bact. Inst., Grahamstown, Cape of Good Hope*, 1905, 27 (Colonial Secretary's Ministerial Division).

bury transmitted it to sheep and goats as also to cattle in 1902. He demonstrated[6] that normally the tick does not carry the virus, but that when it is allowed to feed upon a diseased animal it will pick up the virus, carry it through a moulting period (from larva to nymph or from nymph to adult), and transmit it to the next susceptible host. Lounsbury also discovered a most interesting fact, namely that a larva having obtained the virus and having fed as a nymph upon an insusceptible host is still capable as an adult of infecting its next host, if the host is susceptible. In this respect the virus differs from that of East Coast fever and also in so far that it is not inherited from one generation to another through the eggs.

The period of incubation is about 14 days when the disease is induced by ticks, somewhat shorter after blood inoculation. The febrile reaction is characteristic. The temperature usually rises abruptly to between 105° and 108°F. where it remains for several days, or as long as a week, and then often drops to subnormal before death. Occasionally there are nervous symptoms, such as muscular twitching, tetanic seizures, squinting, excessive salivation, and galloping movements after the animal has fallen to the ground. As Theiler[7] showed in 1903, the symptoms can only be explained on the supposition that we have to do with a microorganism present in the circulating blood. In his opinion the virus does not pass through a Berkefeld or Chamberland filter.[8] The mortality is high (over 50 per cent) and there is no satisfactory method of establishing protective immunity.

At the suggestion of Sir Arnold Theiler that the disease might be due to a *Rickettsia*, a cytological study of the tissues of experimentally infected animals was made in order to test this hypothesis by methods which have proved useful in the investigation of *Rickettsiæ*. It seemed possible that if this theory proved correct information might be secured which would have an interesting though indirect bearing upon the nature of Rocky Mountain spotted fever, typhus fever, and other diseases of man associated with *Rickettsiæ*, the viruses of

[6] Lounsbury, C. P., *Agric. J. Cape of Good Hope*, 1899; 1900, xvii, 682; 1902, xx, 29; xxi, 22, 165, 221, 315; *Rep. Gov. Entomol., 1903*, 15; *Agric. J. Cape of Good Hope*, 1904, xiv.

[7] Theiler, Sir Arnold, *Ann. Rep. Gov. Vet. Bact., 1903–04*, 114; *Vet. J.*, 1904, ix, 300; *Rep. Transvaal Dept. Agric., 1903–04*, 1905, 190; *Ann. Rep. Director Agric., 1904–05*, 1906, 121; *Ann. Rep. Gov. Vet. Bact., 1905–06*, 1907, 67; 1909, 33. Theiler, Sir Arnold, Gray, C. E., and Power, W. M., *10th Internat. Vet. Cong.*, London, 1914. Theiler, Sir Arnold, and Stockman, S., *J. Comp. Path. and Therap.*, 1905, xviii, 155.

[8] Knuth, P., and du Toit, P. J., Tropenkrankheiten der Haustiere, Leipsic, 1921, 628.

TABLE I.

Chart of Animal Passages.

Naturally infected goat from Pretoria district.

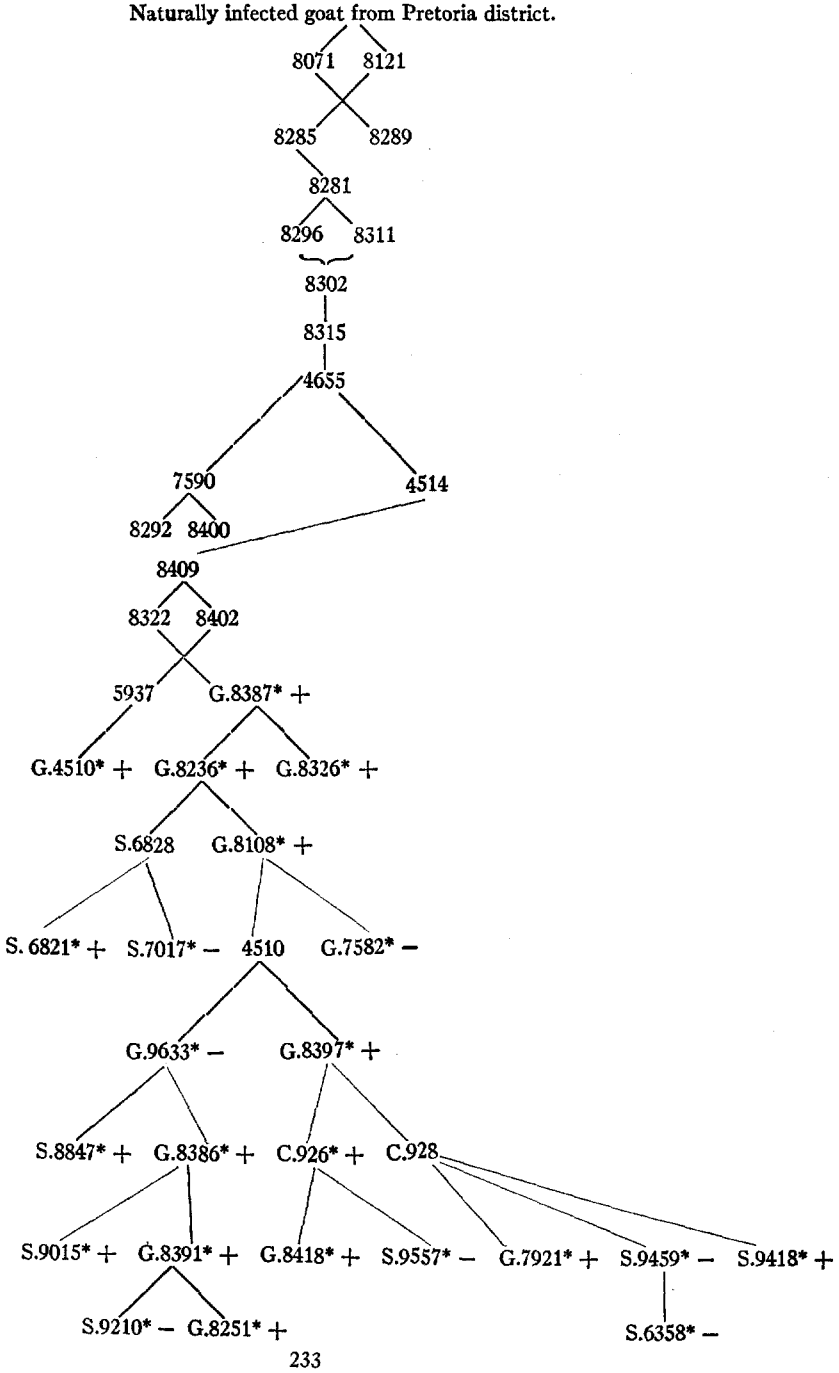


TABLE II.

Summary of Findings.

No.	Inoculated with.	Temperature began to rise on.	Attained maximum on.	Animal died or was killed on.	Pathological changes.	Distribution of microorganisms.
G. 4510	10 cc. blood from G. 5937, no reaction; 10 days later 10 cc. blood from G. 8108.		8th day. 106.8°F.	Killed 14th day. 103°F.	Hydrothorax; marked hydropericardium, fatty degeneration and pigmentation of liver; hyperemia of kidneys and slight enteritis.	Spleen, kidneys, cerebral cortex, cerebellar cortex, midbrain, suprarenal, pancreas, lymph gland, and heart muscle.
G. 7582	10 cc. blood from G. 8108.	7th day.	10th day. 108°F.	Killed 16th day. 105°F.	Anemia. Petechiæ in mucous membrane of trachea and bronchi. Subendocardial petechiæ. Slight hydropericardium and fatty infiltration of liver with pigmentation chiefly in center of the lobules. Tumor splenis. Erosions in mucous membrane of esophagus. Slight infestation with *Hæmonchus contortus*.	None observed.
G. 8108	50 cc. blood from G. 8236.	8th "	12th day. 107.2°F.	Killed 13th day. 107°F.	Hemorrhages under epicardium and left endocardium, emphysema and edema of lungs. Edema and pigmentation of liver (infiltration of interstitium and green pigmentation around central veins). Tumor splenis; interstitial nephritis; slight catarrhal gastroenteritis; larval cysts of *Tænia hydatigena* in omentum and mediastinum; *Trichuris ovis* in cecum.	Spleen, suprarenal, and lymph gland.

G. 8236	10 cc. blood from G. 8387.	11th day.	12th day. 108°F.	Killed 12th day. 105.6°F.	Fibrous pericardial adhesions; tumor splenis; hyperemia of kidneys; stasis and pigmentation of liver; hyperemia of lungs; wireworms in stomach; enteritis; parasitic nodules in omentum; subendocardial hemorrhages in left ventricle.	Spleen.
G. 8326	10 cc. blood from G. 8387.	11th "	12th day. 106.4°F.	Killed 13th day. 105°F.	Slight hydrothorax; edema of lungs; pigmentation and enlargement of liver; degeneration of myocardium and kidneys.	Cerebral cortex.
G. 8386	10 cc. blood from G. 9633.	10th "	13th day. 106.6°F.	Killed 13th day. 106.6°F.	Edema of lungs; areas of fibrosis in liver capsule as a result of perihepatitis chronica; tumor splenis; nephritis (?); slight hydropericardium; caseous lymphadenitis of mediastinal lymph glands; helminthiasis nodularis chiefly in large intestine.	Kidney, cerebral cortex, midbrain, suprarenal, and lymph gland.
G. 8387	10 cc. blood from G. 8322, G. 8402.	7th "	10th day. 107°F.	Killed 12th day. 105°F.	Hydropericardium; epicarditis fibrosa; edema of lungs with marked interstitial edema; chronic cholangitis of intrahepatic bile ducts; *Stilesia hepatica* in tbe lumina; tumor splenis; fatty infiltration of kidneys; few *Hæmonchi contorti* in abomasum; few foci of coccidiosis and *Œsophagostomum columbianum* nodules in intestine. 2 larval cysts of *Tænia hydatigena* attached to serosa of bladder.	Cerebral cortex.

TABLE II—*Continued.*

No.	Inoculated with.	Temperature began to rise on.	Attained maximum on.	Animal died or was killed on.	Pathological changes.	Distribution of microorganisms.
G. 8391	10 cc. blood from G. 8386.	10th day.	11th day. 107.6°F.; excised lymph gland under chloral hydrate.	Died 17th day. 100°F.	Interval after death 7–10 hrs.; hyperemia of lungs; edema and emphysema of right auricle and left endocardium; marked tumor splenis.	Kidney.
G. 8397	10 cc. blood from G. 4510.	10th "	12th day. 106°F.	Killed 12th day. 106°F.	Acute anemia (due to bleeding); slight hydropericardium; degenerative changes in liver and kidneys; tumor splenis; slight nodular worm infestation.	Kidney, medulla oblongata, corpus luteum, and lymph gland.
G. 8418	10 cc. blood from C. 926.	11th "	12th day. 106.4°F.	Died 14th day. 104.2°F.	Interval 1½ hrs.; slight hydrothorax and hydropericardium; petechiæ in left ventricle; fibrous adhesions of liver and diaphragm; tumor splenis; parasitic nodules in large intestine.	Kidney and corpus luteum.
S. 8847	10 cc. blood from G. 9633.	11th "	12th day.	Killed 13th day. 106°F.	Slight hydropericardium; slight edema of lungs; few small calcareous nodules under the capsule; tumor splenis; edema of kidneys; slight infestation with *Hæmonchus contortus*.	Kidney.
G. 9633	10 cc. blood from G. 4510.	5th "	7th day. 107°F.	Killed 8th day. 105.8°F.	Hydropericardium; subendocardial hemorrhages; tumor splenis; wireworm infestation; old *Œsophagostomum* nodules.	None observed.

S. 6821	50 cc. blood from G. 5937.	13th day.	14th day. 106.8°F.	Killed 1[illegible]ay. 105.6°	Slight hydrothorax; slight hyperemia and edema of lungs; infiltration and slight pigmentation of liver; parasitic nodules in liver; subserosal hemorrhages in cecum; *Œsophagostomum columbianum* nodules.	Spleen, kidney, cerebral cortex, and salivary gland.
S. 7017	10 cc. blood from S. 6828.	6th "	10th day. 107.6°F.	14th day normal. Animal recovered. Killed on 30th day.	Hydropericardium; general anemia; *Œsophagostomum columbianum* nodules.	None observed.
S. 9015	10 cc. blood from G. 8386.	10th "	13th day. 107°F.	Died 14th day.	Interval after death 6 hrs. Anemia; sutured wound in left flank; slight hydrothorax and hydropericardium; hypostasis of right lung; fair number of *Œsophagostomum columbianum* nodules in intestine; larval cysts of *Tænia hydatigena* in omentum.	Kidney.
C. 926	20 cc. blood from G. 8397.	11th "	13th day. 106°F.	Killed 14th day. 104°F.	Acute anemia due to bleeding; hydropericardium; edema of lungs; hyperemia of liver; tumor splenis.	Kidney and cerebral cortex.
G. 8251	10 cc. blood from G. 8391.	7th "	Killed 9th day. 103°F.		Slight stasis in lungs; ecchymoses in left endocardium.	Kidney (only a single clump).
G. 7921	10 cc. blood from C. 928.	8th "	12th day. 107°F.	Died 14th day. 103.2°F.	Hydropericardium; hydrothorax; ecchymoses under left endocardium; petechiæ and edema of lungs; stasis and fatty infiltration of liver; marked tumor splenis; slight gastritis.	Kidney, ovary, corpus luteum, cerebellar cortex, corpus striatum, cerebral cortex (organisms not well stained, particularly in kidney and corpus luteum).

TABLE II—*Concluded.*

No.	Inoculated with.	Temperature began to rise on.	Attained maximum on.	Animal died or was killed on.	Pathological changes.	Distribution of microorganisms.
S. 9210	10 cc. blood from G. 8391.	Killed 11th day. 104.8° F.			Slight lymphatic hyperplasia of spleen; *Œsophagostomum columbianum* nodules; *Hæmonchus contortus*.	None observed.
S. 9557	10 cc. blood from C. 926.	10th day.	13th day. 107°F.	18th day temperature normal. 24th day killed.	Parasitic nodules and fatty degeneration of liver; slight fatty degeneration of kidneys; *Œsophagostomum columbianum* nodules in intestine.	" "
S. 9459	10 cc. blood from C. 928.	8th "	11th day. 107.6°F.	17th day temperature normal. 20th day killed.	Hydropericardium; general anemia; infarcts in kidneys; parasitic nodules in intestines.	" "
S. 6358	20 cc. blood from S. 9459. 105° F. Temperature fell to 102° 2nd day.	12th "	Killed 13th day. 104°F.		Tumor splenis, hyperplasia of follicles in spleen; *Œsophagostomum columbianum* nodules; *Ecchinococcus granulosus* in lungs; *Stilesia hepatica* in bile ducts.	" "
S. 9418	20 cc. blood from C. 928.	11th "	15th day. 107.6°F.	Died or killed 17th day. 107°F.	Hydropericardium; slight ascites; degeneration of myocardium; tumor splenis; swelling and fatty infiltration of kidneys; edema of cecum; 2 *Hæmonchi contorti; Œsophagostomum columbianum* nodules; calcified cyst of *Cysticercus tenuicollis;* few foci of coccidiosis in small intestine.	Medulla oblongata and cerebellum.

which, like that of heartwater, have thus far resisted all attempts at artificial cultivation and of which arthropods are the vectors.

Transmission of the Disease.

The heartwater virus employed transmitted the disease with great constancy and was passed through many animals, as shown by the record given in Table I. For convenience each animal was designated by a laboratory number under which heading all information regarding it was assembled. An asterisk (*) indicates that histological examination was made, a plus sign (+) that microorganisms were found, and a negative sign (−) that microorganisms were not observed.

In making the passages infective blood was injected intrajugularly into goats (G.), sheep (S.), and cattle (C.). After a period of incubation of about 10 days the temperature rose suddenly to from 106–108°F. and then subsided, shortly after which the animals usually died or else were killed. Careful autopsies of all were made by the veterinary officers on duty (see Table II). For routine purposes tissues were fixed in Zenker's fluid and were colored by Giemsa's stain, but other special fixatives and stains were also employed as will be mentioned subsequently.

Controls.

The following controls were examined and proved negative for the microorganisms under discussion.

Three sheep and three goats which were held under careful observation for several days before they were killed in order to make certain that they exhibited no signs whatever of disease. For this material I am indebted to Dr. Steck. There were also examined twelve sheep which died from bleeding in the preparation of blue-tongue vaccine; four sheep killed in an advanced stage of jagsiekte;[9] two cattle which died of snotsiekte;[10] and a cow which died of lamsiekte.[11]

[9] Jagsiekte is a contagious chronic catarrhal pneumonia of sheep, the causative agent of which is unknown (Mitchell, D. T., *Rep. Director Vet. Education and Research* (Sir Arnold Theiler), 1915, 585).

[10] "Snotsiekte is an acute specific infectious disease of cattle caused by an ultra-microscopic but non-filterable organism and characterised by a general hyperplasia of lymphoid tissue throughout the body, less frequently by inflammation, erosion and necrosis of the various mucosæ" (Mettam, R. W. M., *Rep. Director Vet. Education and Research* (Sir Arnold Theiler), 1923, 393).

[11] Lamsiekte is a disease of cattle caused by poisoning through the ingestion of a toxin produced by "an anaerobic bacterium reminiscent of, but not identical with, *Bacillus botulinus*" (Theiler, Sir Arnold, Green, H. H., and du Toit, P. J., *J. Agric. Union of South Africa*, 1924, 4).

The Observation of Microorganisms in Animals Experimentally Infected with Heartwater.

At intervals during the febrile period fresh blood was examined by direct and oblique illumination and in smears stained in a variety of ways. No microorganisms were ever found, although it is known that the blood was infective at the time when procured. Fresh smears of various organs taken at autopsy were likewise examined without results. It was only in the fixed and stained tissues that peculiar Gram-negative cocci were found, first in the spleen and later in the other organs. In making the histological examinations it was often necessary to spend hours going over entire sections with the help of a mechanical stage, as is the case also in the demonstration of *Rickettsiæ* in the tissues of animals suffering from Rocky Mountain spotted fever.[12] For this purpose a 1.5 mm. apochromatic objective and a No. 8 compensating ocular were employed.

The microorganisms were most easily detected in the endothelial cells of the capillaries of the renal glomeruli (Figs. 3 and 11) and in the superficial gray matter of the cerebral cortex (Figs. 4, 6 to 8, 10, and 15), which latter, it will be remembered, is the location where Wolbach, Todd, and Palfrey[13] suggested that search be made for the *Rickettsiæ* of typhus fever in experimentally infected guinea pigs. The microorganisms were also found in the following tissues in order of frequency: spleen (Figs. 1 and 2), lymph glands, corpus luteum, cerebellar cortex, suprarenals, midbrain, medulla oblongata, ovaries, corpus striatum, salivary glands, pancreas, and heart muscle. Since they were never found in either the liver or the lungs, it is safe to conclude that in these organs they are either of very rare occurrence or wholly absent. Testicles were seldom available for examination as most of the males had been castrated.

The observation of the microorganisms in the tissues proved to be a rapid and inexpensive method of diagnosis in the case of sheep suffering from anaplasmosis but dying from superimposed attacks of heartwater.

[12] Nicholson, F. M., *J. Exp. Med.*, 1923, xxxvii, 221.

[13] Wolbach, S. B., Todd, J. L., and Palfrey, F. W., The etiology and pathology of typhus, Cambridge, 1922.

It was found that after death the microorganisms remained in the tissues for at least 6 hours. In the case of Sheep 9015 (Table II) they were demonstrated in the kidneys after this length of time, but with considerable difficulty since they had lost their affinity for basic dyes and were recognizable only by their position and morphology. Microorganisms were also detected in fragments of spleen taken from this sheep and kept for a further interval of 11½ hours at room temperature, but there also they were seldom of typical appearance. This early change in the microorganisms runs parallel with the rapid loss of infectivity of the blood and tissues after death.

The Association of the Microorganisms with the Febrile Reaction and with the Lesions.

A very close correspondence was noted between the presence of microorganisms and the febrile reaction. They were never seen in the incubation period, but invaded the endothelial cells before the maximum temperature was reached and as early as 2 days after the initial rise in temperature. It was found that the most favorable time to search for them was when the temperature was subsiding, from 2 to 4 days after the maximum temperature had been reached. They usually persisted, however, for a period of 6 days at the end of which time the temperature had generally fallen to normal. Failure to observe them later than 6 days after the time of maximum temperature was in general accordance with the loss in infectivity of the blood when inoculated into susceptible animals.

Goat 9633 seems to have been an exception in that no microorganisms were found in its tissues although it was killed on the 1st day of the decline in temperature. The incubation period in this case was atypical, being reduced to 5 days, but there was every reason to believe that the animal was actually suffering from heartwater.

The most characteristic lesion and one invariably associated with the presence of the microorganisms was a marked swelling of the endothelial cells. They were seen within the endothelial cells in dense masses varying from a few individuals up to several hundreds (Figs. 1 to 15). The clumps of microorganisms were always found to be surrounded by a halo of cytoplasm staining very lightly or not at all. Isolated microorganisms were never observed. The endo-

thelial enlargements were found in some cases to be so extensive as to entirely block up the lumina of the capillaries—a condition illustrated in Fig. 1. In other cases portions of the cells containing microorganisms became detached and passed into the circulation, or the cells ruptured, with discharge of microorganisms into the blood stream (Fig. 3). The fact, already mentioned, that attempts by many methods to detect the microorganisms in the circulating blood during the febrile reaction were unsuccessful may have been due to the splitting up of these masses of microorganisms into single individuals which, owing to their spherical form, would be difficult to identify in the living state. Moreover, in fixed preparations the stain would be very easily extracted during differentiation on account of their small size.

Except for the presence of the microorganisms the cytoplasm of the endothelial cells showed no deviation from the normal and it seldom contained recognizable products of the phagocytosis of hemoglobin or fatty inclusions, or any of the basophilic granulations reported in typhus fever.

Neighboring endothelial cells devoid of microorganisms appeared normal and were not enlarged. No evidence was found of undue multiplication of the endothelial cells. No thrombosis was noted and the microorganisms were never seen in association with any kind of leucocytic infiltration or in any extravascular location. In other words, they seemed to excite no detectable local tissue reaction other than the physical distention of the cells to accommodate them in large number.[14]

The Morphology of the Microorganisms.

The microorganisms were found to be very uniform coccus-shaped bodies, 0.2 to 0.5 μ in diameter, as measured after fixation in Zenker's fluid or Regaud's fluid and coloration by Giemsa's stain, or by any basic aniline dye. Their spherical shape is well shown in Figs. 1 to 6. No agglutinations or marked irregularities in morphology suggestive of intracellular digestion were observed. In a single clump the

[14] The histopathology of heartwater will soon be made the subject of a special paper by Dr. Steck, who has also enjoyed the privilege of working in Sir Arnold Theiler's laboratory.

microorganisms were, so far as could be seen, of the same size; but adjacent clumps in the same section occasionally differed slightly in the size of the individuals composing them, within the limits specified above and as represented in Fig. 1. The microorganisms had the appearance of being slightly larger when present in small masses and when not closely crowded together, as in the case of accumulations of many hundreds; but the difference was not sufficiently marked to exclude the possibility that it may have been merely an optical illusion. Occasionally they were observed in diplo formation (Fig. 11), but this was the exception rather than the rule. No morphological evidence was detected of any multiplicative phase, other than this diplo formation indicating the likelihood of simple division. The shape and size of the microorganisms remained constant throughout the febrile reaction.

Microchemical Reactions of the Microorganisms.

The microorganisms were well preserved after fixation in Regaud's fluid[15] and Zenker's fluid both with and without acetic acid, but all the fixatives commonly used for bacteria were suitable. They were colored deep, clear blue by Giemsa's method. Those in diplo formation showed no trace of red-staining material between the two halves such as has often been described in *Rickettsiæ* in lice; but sometimes they exhibited halos. They were likewise easily stained by Löffler's methylene blue and other basic aniline dyes. When treated with Unna-Pappenheim's methyl green-pyronine mixture, they usually stained light red, but sometimes acquired a slightly greenish tint, depending upon the method of staining. They were Gram-negative and stained readily with fuchsin, but did not retain it on differentiation. They were nicely stained with iron-hematoxylin, but on differentiation became bleached before the nuclear chromatin and erythrocytes. Some resisted decolorization more than their neighbors and remained jet-black while the others became light gray, but this may have been occasioned by irregular washing out of the mordant (iron alum) and may not indicate the existence of a true qualitative difference *inter se*. When autolysis of the tissue was

[15] 4 parts of a 3 per cent solution of potassium bichromate and 1 part of commercial formalin—a fixative intended primarily for the study of mitochondria.

allowed to proceed their affinity for basic dyes was lost before that of the nuclear chromatin.

Distinction from Normal Cellular Components and the Products of Degeneration and Phagocytosis.

Although the association of the microorganisms with heartwater, their morphology, and their staining reactions were found to be so definite, it was decided to compare them carefully with normal cellular components and with the products of degeneration and phagocytosis, since, up to the present time, like most *Rickettsiæ*, their status as living organisms has not been proved by methods of artificial cultivation.

When they were first detected in the spleen (Figs. 1 and 2) the possibility of confusion with some unfamiliar granular type of blood or bone marrow cell was carefully considered on account of their characteristic tendency to occur in dense clumps, but this was soon definitely excluded by the observations, already alluded to, that they seemed to be identical in three species (*i.e.* goats, sheep, and cattle); that they occurred only in a certain phase of the febrile reaction, being absent in controls; and that, in distribution, they were restricted to the cytoplasm of endothelial cells, being surrounded by a distinct zone of rarefaction. Another point of distinction was that the microorganisms were always disposed in clumps at one side of the nucleus. The nucleus was never surrounded on all sides by them as is usually the case with the specific granules in granular leucocytes. Although a search was made of the bone marrow, no cells containing granules at all resembling them were found.

Unlike mitochondria, they were readily preserved by Zenker's fluid containing the usual 5 per cent of acetic acid. They were basophilic, whereas the mitochondria are acidophilic. In shape also they were different from mitochondria, because no rod-like or filamentous forms were seen. They were present in the cytoplasm in large and compact masses in contrast to the mitochondria which are never so abundant and are not grouped in the same way. Finally, by their restriction to the endothelium, they were clearly differentiated from mitochondria and all mitochondrial products.[16]

The microorganisms were also distinguished from the granules of mast cells by their uniformity in size and shape, by the fact that they generally stained clear blue, instead of purple, by Giemsa's method, and similarly by their restriction

[16] The distinction between mitochondria and bacteria is discussed by Cowdry and Olitsky (Cowdry, E. V., and Olitsky, P. K., *J. Exp. Med.*, 1922, xxxvi, 521), and between mitochondria and the *Rickettsia* of Rocky Mountain spotted fever by Nicholson.[12]

to the cytoplasm of endothelial cells (see Fig. 5). They were never found outside the blood vessels.

Neither were the microorganisms to be confused with products of the phagocytosis of hemoglobin—an explanation of the nature of *Rickettsiæ* advanced by Woodcock.[17] In the first place, phagocytosis of fragments of red blood cells by the endothelium was not a process commonly met with in heartwater. In the rare instances in which it did occur the resultant inclusions took the form of droplets grading from about the size of a red blood corpuscle down to a few microns in diameter and stained much more strongly than did the microorganisms with iron-hematoxylin. When colored by Giemsa's method, these inclusions frequently exhibited a yellowish or greenish tinge, due to the superposition of pigment and stain. They were never clear blue like the microorganisms, nor were they clumped in so characteristic a fashion.

The microorganisms stained much the same color as nuclear chromatin, but were easily differentiated from it or any of its degenerative products. As is indicated by the figures, the nuclei of the endothelial cells containing them seldom revealed any traces of division or degeneration so that chromatin was not being emitted in a form detectable morphologically. It was to be noted that except for the presence of the microorganisms the cytoplasm of the endothelial cells showed no modifications microscopically visible. In this respect the condition of the vascular endothelium in heartwater differed sharply from that found in typhus fever as described by Wolbach and his associates.[13,18]

DISCUSSION.

It is interesting to compare the above mentioned microorganisms with *Rickettsiæ*, but to do so consistently is by no means a simple matter because there is so little unanimity of opinion as to what distinguishing features *Rickettsiæ* actually possess.

If the suggestion, made in an earlier paper,[19] that in the identification of *Rickettsiæ* stress may properly be placed upon "the ability of the organisms to lead an intracellular existence, their location in the tissues, their host specificity, their Gram-negative properties, and their bacterium-like morphology" be accepted by others, as it has been by Hertig and Wolbach,[20] we are clearly justified in including these microorganisms in the general category of *Rickettsia*. These authors, in practice have, however, slightly modified the definition to read as follows:

---

[17] Woodcock, H. M., *J. Roy. Army Med. Corps*, 1921, xxxvii, 418; 1922, xxxix, 243; 1923, xl, 81, 241; 1924, xlii, 175.

[18] Wolbach, S. B., and Todd, J. L., *Ann. Inst. Pasteur*, 1920, xxxiv, 153.

[19] Cowdry, E. V., *J. Exp. Med.*, 1923, xxxvii, 431.

[20] Hertig, M., and Wolbach, S. B., *J. Med. Research*, 1924, xliv, 329.

TABLE III.

Comparison of the Rickettsiæ of Rocky Mountain Spotted Fever and of Typhus Fever with the Microorganisms in Heartwater as Seen in Mammalian Tissues.

	Spotted fever.	Typhus fever.	Heartwater.
Morphology.	(*a*) Paired; 0.2 to 0.3 by 1 μ; often surrounded by halo. (*b*) Rod-like forms 1 μ in length and often possessed of polar granules. (*c*) Rounded forms.[23]	(*a*) Ovoid, somewhat lanceolate bodies in pairs. The pairs measure slightly over 1 by 0.2 to 0.3 μ. (*b*) Smaller coccoid bodies.[13]	Very uniform coccoid bodies 0.2 to 0.4 μ in diameter, rarely in pairs.
Phases of multiplication.	None detectable in mammalian tissues other than possibly simple division.	The same.	The same.
Microchemical reactions.	Best seen after fixation in Zenker's fluid and coloration by Giemsa's stain. Easily stained by basic aniline dyes, Gram-negative, and not acid-resistant.	The larger paired bodies stain more readily than the cocci and are surrounded by a slight halo.[13] Gram-negative and not acid-resistant.	Stain more easily, otherwise the same.
Detection.	Not visible, or visible with difficulty, in living cells unstained.	Not reported in living cells.	Not seen in living cells.
	Demonstrated in teased cells.[23]	Not reported in teased cells.	Not as yet seen in teased cells.
	Occurs in blood in exceedingly small numbers (Ricketts confirmed by Wolbach[23]).	Not as yet clearly seen in blood.*	Observed occasionally within vascular lumen in sections but not in blood smears.
	Detectable in sections after careful search.	More difficult to detect in sections than spotted fever organism.	Easier to observe in sections than either of the others.

Position.	Chiefly within the cytoplasm of endothelial cells, but also in the smooth muscle of the media;[13] in endothelial cells which collect in and around the adventitia;[23] in vascular lumina, giant cells, liver cells, and mononuclear leucocytes.[12]	Restricted to the cytoplasm of endothelial cells.†	Definitely restricted to the cytoplasm of endothelial cells and to portions of them broken off into the vascular lumen. Occasionally the cells rupture and discharge single microorganisms into the blood stream.
Arrangement.	Single and in clumps of varying size.	"Globular massing of organisms is the most characteristic appearance of *Rickettsia* in human lesions."[13]	Spherical clumps are more marked than in either spotted fever or typhus, and attain a much larger size. They are often multiple, several discrete clumps being present within a single endothelial cell.‡
Distribution.	Skin, scrotum, epididymis, testis, thyroid, spleen, lungs, and skeletal muscle[24] and in addition in heart, adrenals, lymph glands, and liver.[12]	Skin in 27 cases, kidneys in 2, femoral vein in 1, testes and adnexa in 5, and brain in 7.[13]	Kidney in 11 cases, cerebral cortex in 7, spleen in 4, lymph glands in 4, cerebellar cortex in 3, suprarenals in 3, corpus luteum in 3, midbrain in 2, medulla oblongata in 2, pancreas in 1, heart muscle in 1, corpus striatum in 1, ovary in 1, salivary glands in 1.

* According to Wolbach, Todd, and Palfrey[13] Ricketts' observation of bipolar microorganisms in the blood of typhus fever patients has not been confirmed.

† On page 189 Wolbach, Todd, and Palfrey[13] state that: "they [*Rickettsiæ*] are found only in the endothelium and never, as in Rocky Mountain spotted fever, in the smooth muscle of the media." On the next page, however, they refer to the finding of *Rickettsiæ* in the mononuclear cells of the perivascular nodules. On page 192 the localization of *Rickettsia* in endothelial cells is again emphasized.

‡ In certain of their morphological features these clumps resemble the so called trachoma bodies described by Noguchi and Cohen (Noguchi, H., and Cohen, M., *J. Exp. Med.*, 1913, xviii, 572, Fig. 1).

TABLE III—*Concluded.*

	Spotted fever.	Typhus fever.	Heartwater.
Association with febrile reaction.	Increase in number progressively with development of the lesions from 1st to 5th or last day of fever when both attain a maximum. Early in reaction diplobacillary becoming bacillary in later stages.[12]	Found in every case (*i.e.* 25) where postmortem examination was made before the 13th day of the disease and while the body was in a fresh condition.[13]	Found in 16 cases, most frequently within the 6 day period after the temperature has commenced to decline.
Association with lesions.	Direct injury caused by parasite shown by degenerative changes in endothelial cells and smooth muscle cells of media.[23]	Similar swelling and degeneration of endothelial cells.	Endothelial cells swollen, but, in contrast, show no sign of degeneration.
	Thrombosis, necrosis, and perivascular infiltration chiefly in subcutaneous tissue associated with the development of a rash.	Similar.	No thrombosis, necrosis, or perivascular infiltration and consequently no rash. The most characteristic lesion is a variable degree of hydropericardium.

"Gram-negative, intracellular, bacterium-like organisms found in arthropods." This inclusion of the word "arthropod" necessitates mention of the fact to be reported in a succeeding paper that similar microorganisms have been found in the insect vector of heartwater[21] so that the *Rickettsiæ* under consideration fulfill this condition also. My discussion will be limited to the microorganisms as seen in mammalian tissues.

In a recent critical review of "the position of *Rickettsia* as an ætiological factor in disease," Arkwright[22] expressed the opinion that *Rickettsiæ* are only known to be concerned with three diseases; namely, Rocky Mountain spotted fever, typhus fever, and trench fever. They have been frequently seen in the invertebrate ectoparasitic hosts in association with all three. In mammalian tissues they have also been reported in Rocky Mountain spotted fever, chiefly by Wolbach[23, 24] and in typhus fever by Kuczinski,[25] by Wolbach and Todd,[18] by Stevenson and Balfour,[26] with certain reservations, and more emphatically by Wolbach, Todd, and Palfrey;[13] but it is questionable whether they have been observed in trench fever.

The kindness of Professor Wolbach and of Dr. Nicholson in giving me slides containing the *Rickettsia* of spotted fever, and of Professor Wolbach in giving me tissues from cases of typhus fever, which I have sectioned and stained myself, makes it possible for me to compare these *Rickettsiæ* very closely with the microorganisms in heartwater. For convenience this comparison is given in tabular form. Rocky Mountain spotted fever is listed first as the only disease in which *Rickettsiæ* have been proved to be the etiological agents and in which they may invariably be detected in the tissues; then typhus fever, in which Wolbach and his colleagues have presented valuable evidence in favor of *Rickettsiæ* as the causative agents; and lastly heartwater.

In addition to this close resemblance between the three microorganisms as brought out in Table III, there are certain points of similarity between the viruses of the three diseases. In each case the virus may be transmitted by the bites of infective insects (or arachnids) or by the inoculation of infective blood in which *Rickettsiæ* are not demonstrable or may be seen with difficulty (Rocky Mountain spotted fever). All three occasion a high temperature reaction and a heavy mor-

---

[21] Cowdry, E. V., *J. Exp. Med.*, 1925, xlii, 253.

[22] Arkwright, J. A., *J. Roy. Army Med. Corps*, 1924, xlii, 447.

[23] Wolbach, S. B., Rocky Mountain spotted fever, in Bryam and Archibald, The practice of medicine in the tropics, Oxford Medical Publications, Oxford, 1923, iii, 2092.

[24] Wolbach, S. B., *J. Med. Research*, 1919–20, xli, 1.

[25] Kuczinski, *Centr. allg. Path. u. path. Anat.*, 1919–20, xxx, quoted from Arkwright, J. A., Typhus fever, in Bryam and Archibald, The practice of medicine in the tropics, Oxford Medical Publications, Oxford, 1923, iii, 2078.

[26] Stevenson, A. C., and Balfour, A., *J. Path. and Bact.*, 1921, xxiv, 289.

tality. The virus of heartwater seems to differ from that of Rocky Mountain spotted fever and to resemble that of typhus fever in respect to the fact that it is not inherited through successive generations in the eggs of the invertebrate vector. All three viruses are unfilterable and as yet uncultivable.[27] They do not retain their vitality even under the most favorable conditions *in vitro* for more than a few days; and one attack of the disease confers a permanent immunity or one lasting for several years.

A divergence in clinical symptoms between heartwater, on the one hand, and spotted fever and typhus fever, on the other, is to be expected, because heartwater is, by contrast, a disease of ruminants only. In all three, however, functional disturbances of the nervous system are of common occurrence. The absence of a rash in heartwater is probably due to the non-involvement of the cutaneous blood vessels. It is this property, that the vascular endothelium in all parts of the body retains its normal vitality and is not injured by the action of the virus, which makes heartwater so very favorable for the study of the general problem of the relation of *Rickettsiæ* to disease. In heartwater the *Rickettsiæ* may be easily studied in endothelial cells which are to all appearances normal except for the mechanical distention which they have undergone to accommodate the *Rickettsiæ* in large numbers. By contrast in both spotted fever and typhus fever the blood vessels are the seat of severe lesions and the endothelium is much involved. This injury results in the appearance of numerous granules within the endothelial cells, which mask the microorganisms. In consequence of this fact investigators have been slow to accept conclusions based wholly upon the histological study of *Rickettsiæ*, particularly so since the *Rickettsiæ* of both spotted fever and typhus have resisted many and repeated attempts at artificial cultivation. Not only is the study of heartwater relieved from this handicap, but in addition the *Rickettsiæ* may be rapidly demonstrated by relatively simple methods of technique.

SUMMARY.

A Gram-negative, intracellular, coccus-like microorganism was found in cases of heartwater in the three species which are susceptible to the disease; namely, goats, sheep, and cattle. It was absent in the case of control animals, both normal ones and those dying of some other diseases. The presence of this microorganism was definitely related to the febrile reaction. It was most easily detected in the renal glomeruli and in the small capillaries of the cerebral cortex but probably occurred throughout the body. The microorganism

[27] In this respect they apparently differ from (1) *Rickettsia melophagi* (Nöller, W., *Arch. Schiffs- u. Tropen-Hyg.*, 1917, xxi, 53); (2) *Rickettsia rocha-limæ* (Weigl, R., *Przeglad Epidemjol.*, 1921, i, 373); and (3) *Rickettsia nipponica* (Sellards, A. W., *Am. J. Trop. Med.*, 1923, iii, 529).

was a typical endothelial parasite, being restricted in distribution to the endothelial cells of the smaller blood vessels and to portions of such elements which had broken off into the blood stream. It was never observed to cause injury to the cells other than those incident to mechanical distention through accumulation within them of many individuals in large densely packed masses which were characteristically spherical. A typical attribute was the presence of several of these masses within the cytoplasm of a single endothelial cell. In view of the association of this microorganism with heartwater, a disease of ruminants, and thus far the only one in which microorganisms resembling *Rickettsiæ* have been reported, the designation *Rickettsia ruminantium* is proposed.

EXPLANATION OF PLATES.

PLATE 10.

The microorganisms in the endothelial lining of small blood vessels are represented as colored with Giemsa's stain. The drawings were made with Zeiss apochromatic objective 1.5 mm., compensating ocular 8, and camera lucida giving a magnification of 1,940 diameters.

FIG. 1. Spleen (G. 4510) fixed in Regaud's fluid. A small branching capillary is shown, the lumen of which is occluded by the enlargement of endothelial cells containing microorganisms.

FIG. 2. Spleen (S. 6821) fixed in Zenker's fluid. A swollen endothelial cell is depicted containing a roughly spherical mass of microorganisms embedded in chromophobic cytoplasm.

FIG. 3. Kidney (G. 4510) fixed in Regaud's fluid. A clump of microorganisms is seen discharging into the blood stream.

FIG. 4. Cerebral cortex (G. 8386) fixed in Zenker's fluid. A clump of microorganisms in the endothelial lining of a small blood vessel.

FIG. 5. Kidney (G. 4510) fixed in Regaud's fluid. An endothelial cell containing two masses of microorganisms with the lumen (at the base of the drawing) almost blocked. In contact with it is an histogenous mast cell possessing granules which are of irregular size and shape and colored deep red.

FIGS. 6 and 7. Cerebral cortex (G. 4510) fixed in Zenker's fluid. Two capillaries with endothelial cells containing clumps of microorganisms surrounded by zones of unstained cytoplasm.

FIG. 8. The same. A rather larger blood vessel with two endothelial cells in different stages of engorgement.

FIG. 9. Kidney (G. 4510). A still larger blood vessel with engorged endothelial cells in which the microorganisms are arranged in multiple sharply outlined clumps which were found to be very characteristic of heartwater.

PLATE 11.

The photomicrographs were taken with Zeiss apochromatic objective 3 mm., 1.40 aperture, and compensating ocular 8 giving a magnification of 1,400 diameters. The preparations were fixed in Zenker's fluid and colored by Giemsa's stain.

FIG. 10. A small blood vessel in the cerebral cortex (G. 4510) in which two clumps of microorganisms are seen within the cytoplasm of endothelial cells. The cell containing the largest clump completely occludes the lumen of the vessel. There is a smaller clump in the next endothelial cell to the left, colored more intensely, since the microorganisms are more closely packed together. Each clump is encircled by a halo of lightly stained cytoplasm.

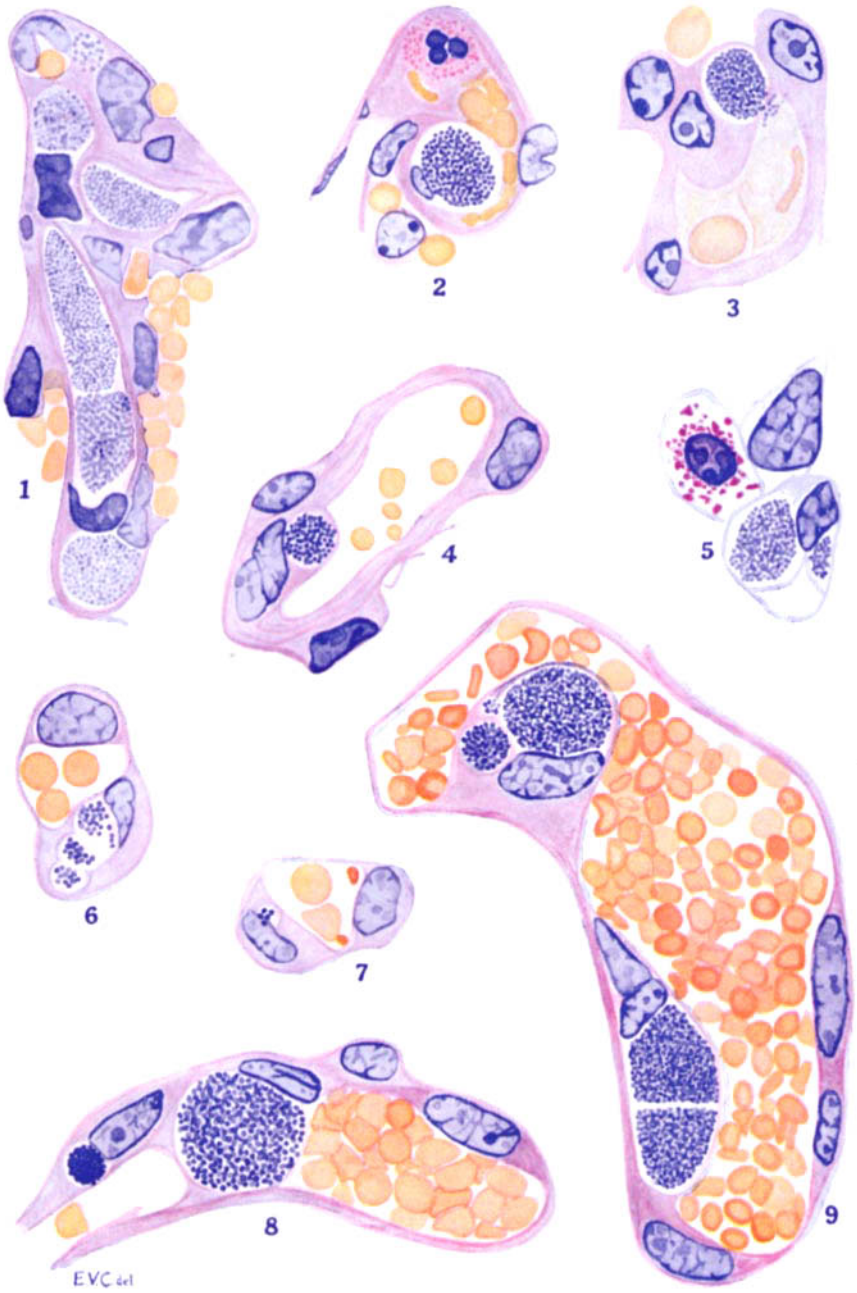
FIG. 11. A clump of microorganisms within an endothelial cell of a renal glomerulus (G. 8386). In some places it may be seen that the organisms are grouped in pairs and that each is surrounded by a halo.

FIG. 12. Four clumps of microorganisms within an endothelial cell of a capillary between the renal tubules (G. 4510). The fourth clump on the left is slightly out of focus. The nucleus is flattened and lies just below the microorganisms.

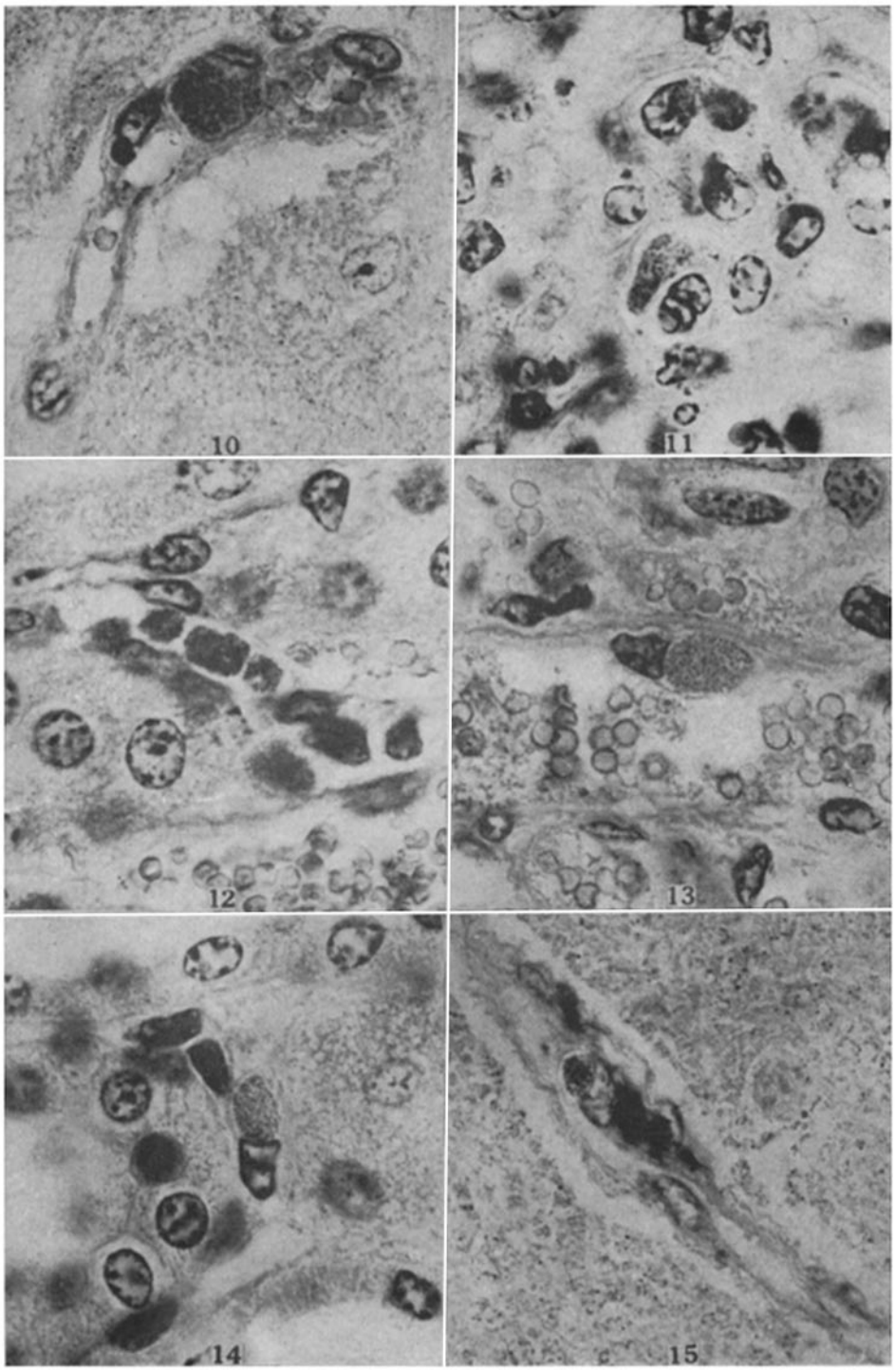
FIG. 13. A clump of microorganisms in an endothelial cell of a somewhat larger blood vessel of the same kidney.

FIG. 14. Another group of microorganisms within an endothelial cell of a capillary of the same kidney.

FIG. 15. A clump of microorganisms in an endothelial cell of a small blood vessel of the cerebral cortex (G. 8386).



(Cowdry: Etiology of heartwater. I.)



(Cowdry: Etiology of heartwater. I.)